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Targeted genome editing in tomato: Unraveling gene regulation in carotenoid biosynthesis

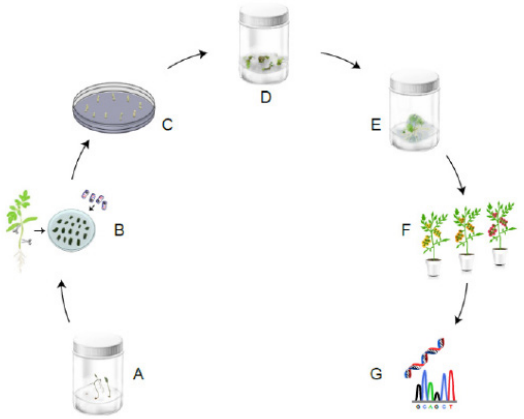
Overview

Signios Bio leveraged its expertise in plant genomics to conduct a proof of concept study on carotenoid biosynthesis in tomatoes using CRISPR/Cas9 technology, published in Plant Cell Reports[1]. The research demonstrates that a controlled knockdown of genes can be achieved by targeting the regulatory regions, rather than coding regions that often have unwanted consequences of knockdown.

Challenge

Understanding the role of Carotenoid Isomerase (*CRTISO*) in tomato fruit coloration posed a key challenge. While previous studies focused on gene knockouts, this research aimed to:

- Compare the impact of targeted deletions in the coding region vs. the 5' UTR (Untranslated Region).
- Assess how these edits influence gene function and fruit phenotype, particularly lycopene production.



Workflow for genome editing in *PKM1* tomato plants from Lakshmi Jayaraj, K., *et al.* (2021).

Solution delivered

Signios Bio applied the following solutions:

1. **CRISPR/Cas9 genome editing:**
   - Designed and deployed guide RNAs to target both exon 1 and the 5' UTR of the *CRTISO* gene in *Solanum lycopersicum.*
   - Used Agrobacterium-mediated transformation to successfully deliver CRISPR/Cas9 constructs into tomato explants and regenerate plants.

2. **Advanced molecular analysis:**
   - **Mutation confirmation:** PCR and Sanger sequencing validated successful edits.
   - **Gene expression analysis:** RNA-seq and qPCR assessed the effects of edits on *CRTISO* expression levels.
   - **Phenotypic characterization**: HPLC profiling quantified lycopene and carotenoid content in mutant fruits.

3. **High-quality bioinformatics support:**
   - Analyzed transcriptome data to determine gene expression changes and confirm the regulatory effects of the 5' UTR deletion.

Results

The study revealed critical insights into *CRTISO* regulation and function:

1. **Exon editing (knockout):**
   - Mutations in exon 1 resulted in a complete loss of function of the CRTISO enzyme.
   - Plants displayed a tangerine phenotype, with a 98% reduction in lycopene levels, pale-yellow fruits, and significant changes in leaf chlorophyll content.

2. **5' UTR editing (knockdown):**
   - Deletions in the 5' UTR caused downregulation (but not knockout) of *CRTISO* expression.
   - Fruits showed an intermediate phenotype (lighter red), with reduced lycopene content, while maintaining functional enzyme activity.

3. **Key discovery:**
   - The 5' UTR contains essential cis-regulatory elements that control *CRTISO* gene expression. Editing this region allows researchers to fine-tune gene expression and achieve phenotypic modulation without full gene disruption.

Sanger sequencing chromatogram comparing the sequence of wild type *PKM1* to the homozygous mutants obtained by segregation of the biallelic heterozygous plant #42 and homozygous mutant #58.7 from Lakshmi Jayaraj, K., *et al.* (2021).

Fruit phenotype comparison between WT, Homozygous T2 plants of Exon mutant (#42.1.20 and 5'UTR mutant (#58.7.3) from Lakshmi Jayaraj, K., *et al.* (2021).

Impact and value

By combining genome editing precision with molecular and bioinformatics analysis, Signios Bio successfully:

- Demonstrated the power of targeted genome editing in crop functional genomics.
- Highlighted how regulatory region edits offer a strategic approach for crop improvement without compromising plant robustness.
- Contributed critical knowledge to carotenoid biosynthesis pathways, a key trait for both agricultural productivity and human nutrition.

This study underscores the combined expertise of Signiosbio in providing end-to-end plantgenomics solutions, enabling researchers to uncover genetic mechanisms and drive meaningful agricultural innovation.

Reference

1. Lakshmi Jayaraj, K., *et al.* Targeted editing of tomato carotenoid isomerase reveals the role of 5′ UTR region in gene expression regulation. *Plant Cell Rep* 40, 621–635 (2021). https://doi.org/10.1007/s00299-020-02659-0

About Signios Bio

Signios Bio offers world-class multiomics solutions for plant and animal genomics research. From genome editing to high-throughput sequencing and advanced bioinformatics, we empower scientists to accelerate discoveries, improve crop traits, and address global agricultural challenges.

Looking to explore genome editing or plant genomics solutions?

Contact us to collaborate.